Comments to the National Toxicology Program (NTP) Review Panel on the draft NTP technical report entitled "TOXICOLOGY AND CARCINOGENESIS STUDIES OF ALPHA-METHYLSTYRENE (CAS NO. 98-83-9) IN F344/N RATS AND B6C3F1 MICE (INHALATION STUDIES)"

These comments reflect review of the toxicology and carcinogenesis studies of Alpha methylstyrene (AMS) in rats and mice (inhalation studies). The hope is for the expert panel to consider these comments in the final report.

Critical Points:

- A thorough review of the NTP testing results from both the 2-year and the 3-month inhalation studies on AMS support an alpha-2-u-globulin nephropathy (α2u-N) mediated mechanism for the induction of kidney tumors in male rats. The induction of nephropathy and subsequent tumor formation is weak when compared to a known inducer, decalin.
- The incidence of hepatocellular adenomas and carcinomas in the chamber control was lower than the mean of the historical control in B6C1F1 female mouse inhalation studies. This may account for the apparent statistically significant tumor increase observed in female mice.
- NTP data indicates that in the chronic study, the Maximum Tolerated Dose (MTD) as defined by NTP was exceeded in certain groups of male and female mice which confounds the interpretation of study results.

AMS is an inducer of alpha-2-u-globulin nephropathy

In 1991 EPA published a review of all the data supporting this mechanism of action and the data to support that this pathway was not relevant to humans. Also outlined were the criteria that needed to be fulfilled in the process of distinguishing this mechanism of renal tumors from other potential mechanisms. AMS meets these criteria, and is considered to be a weak α2u-globulin inducer by NTP (2006). With weak inducers, typically not all aspects of the pathological sequence of lesions are observed, and this is true for AMS.

Table 1. Kidney adenoma and carcinoma (combined) data for 2-year inhalation exposures to AMS or Decalin.

AMS Single Section	AMS Step Section	Decalin Single Section
0/50 (0 ppm)	1/50 (0 ppm)	1/50 (0 ppm)
0/50 (100 ppm)	2/50 (100 ppm)	3/50 (25 ppm)
2/50 (300 ppm)	3/50 (300 ppm)	7/50 (50 ppm)
2/50 (1000 ppm)	7/50* (1000 ppm)	12/50* (100 ppm)
		6/20* (400 ppm)

Table 1 represents kidney adenoma and carcinoma (combined) data for 2-year inhalation exposures to either AMS or decalin. When single sections of the kidney were examined after AMS exposure, findings were not statistically significant. However, when step

sections were taken, incidences of renal tubule adenoma and carcinoma (combined) in the 1000 ppm male rats were significantly greater than those in chamber controls.

If this data is compared to a stronger inducer of α2u-globulin nephropathy, decalin, one observes that the renal tumor response in AMS exposed male rats is weak in comparison. Note that the decalin incidence is measured after single sectioning.

Dose-response relationships for end-points characteristic of α2u-Nephrophathy, such as protein droplets, α2u accumulation and cell proliferation, are similar for AMS and decalin (Figure 1). The left figure represents NTP data from 3 month studies with Decalin and AMS. The pound symbol represents the exposure concentration in which renal tumors were found to be significant in the 2 year study. It is critical to note that renal tumors were not observed following concentrations of both decalin and AMS in which there was a significant increase in labeling index, protein droplets, and α2u concentration, although this was stated in the AMS report (NTP, 2006) to be a reason why other mechanisms besides α2u-nephropathy must be operative for the renal tumors induced by AMS following 1000 ppm exposures.

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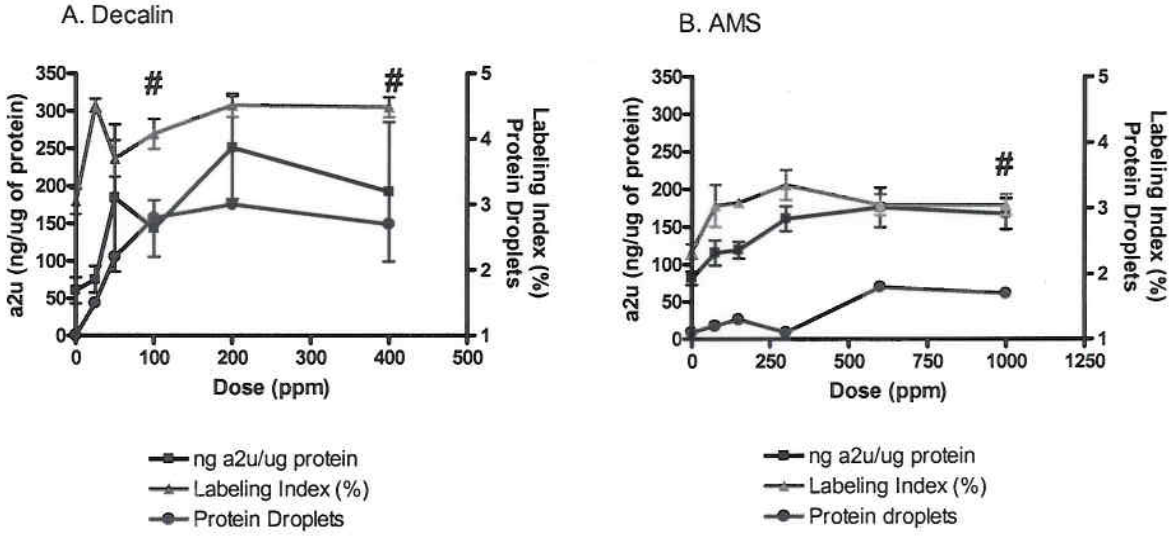
Figure 1. Dose-responses for end-points characteristic of α2u-N are similar in AMS and Decalin 3-month exposures



The control liver tumor incidence in female mice was low

The incidence of hepatocellular adenomas and carcinomas in the chamber control was lower than the mean of the historical control in B6C1F1 female mouse inhalation studies (Table 2). This may account for the apparent statistically significant tumor increase observed in female mice.

Table 2. Incidences of Liver Lesions in Female Mice in the 2-Year Inhalation Study of AMS

	Historical Control	Chamber Control	100 ppm	300 ppm	600 ppm
Incidence of Hepatocellular Adenoma or Carcinoma	31.1%±6.8	26%	52%	48%	66*%

The MTD may have been Exceeded in Male and Female Mice

In the NTP, the maximum tolerated dose (MTD) is defined as the highest dose that does not cause >10% decrease in body weight gain and survival relative to the controls during the study or a toxic lesion of a severity that would confound the study.

There was no biologically significant change in body weight gain in either male or female rats during the 2-year exposure, although the body weight in both sexes at the highest dose was decreased for a long period starting at about 21 weeks. However, the decrease was not at a level to argue that the highest dose exceeded the maximum tolerated dose (MTD). In contrast, the body weights relative to controls of both male and female mice were significantly decreased starting at about 16 weeks. The magnitude of the difference exceeded the MTD for a majority of the study in the 600 ppm dose males and at 300 and 600 ppm dose females. This may confound mouse findings.

Figure 2. Female Mouse Body Weight Changes in 2-year study with AMS

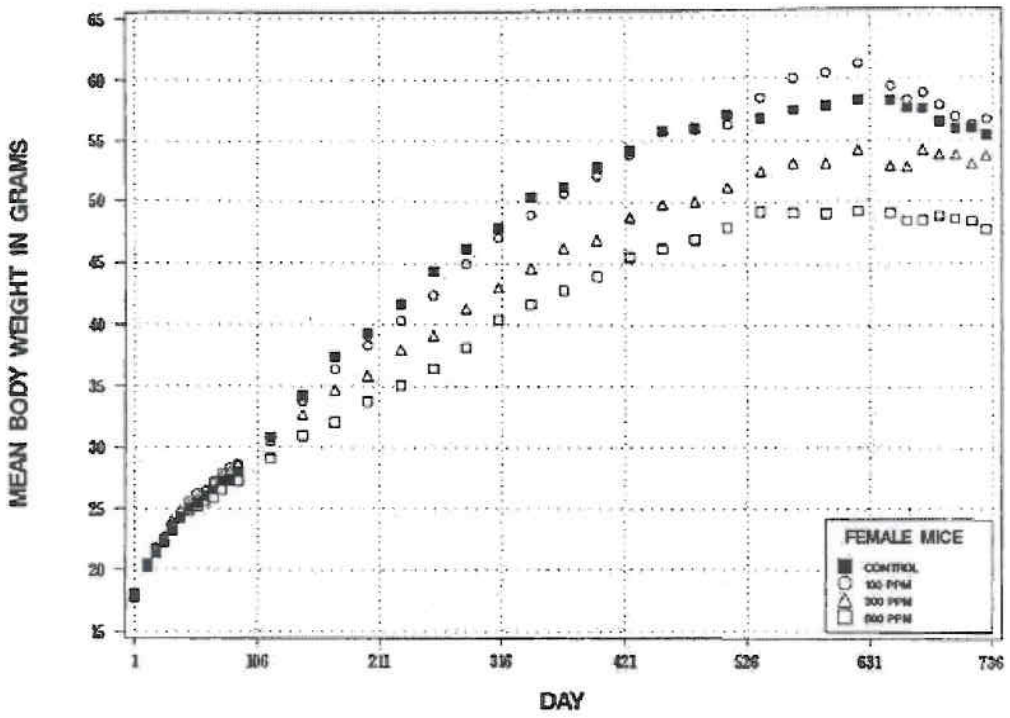
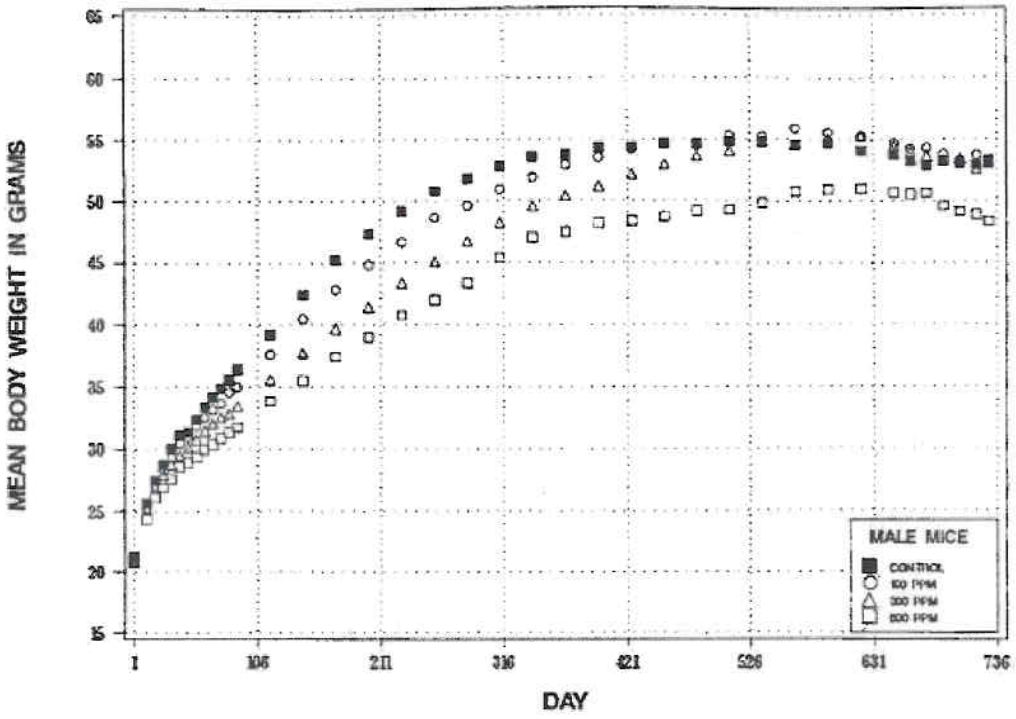


Figure 3. Male Mouse Body Weight Changes in 2-year study with AMS



In conclusion,

- A thorough review of the NTP testing results from both the 2-year and the 3-month inhalation studies on AMS support an alpha-2-u-globulin nephropathy (α2u-N) mediated mechanism for the induction of kidney tumors in male rats. The induction of nephropathy and subsequent tumor formation is weak when compared to a known inducer, decalin.
- The incidence of hepatocellular adenomas and carcinomas in the chamber control was lower than the mean of the historical control in B6C1F1 female mouse inhalation studies. This may account for the apparent statistically significant tumor increase observed in female mice.
- NTP data indicates that in the chronic study, the Maximum Tolerated Dose (MTD) as defined by NTP was exceeded in certain groups of male and female mice which confound the interpretation of study results.

It is requested that these points be considered in the final report.